Organization: Stanford University

Title: Quantitative Development of Biomolecular Databases, Measurement Methodology, and Comprehensive Transport Models for Bioanalytical Microfluidics

MTO **Simbiosys**

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Project Goals

The goal of this project is the development of fully-validated, multidisciplinary models for the design of microfluidic bioanalytical systems. The design and simulation of robust, field-portable microfluidic devices for the detection of biological weapon agents requires a detailed understanding of the fundamental transport and biochemical reaction/binding processes associated with bioanalytical systems. These processes include sample handling, mixing, incubation, separation, diffusion, and bio-reaction and molecular binding processes. High-fidelity modeling of transport and biomolecular reaction kinetics requires methods of building a data base of empirical flow parameters and molecule-specific data.

Technical Approach:

The project incorporates an interactive approach that leverages experiments, simulation, and fabrication so that each step of the experiment data acquisition, flow experiment interrogations, and ligand-receptor/flow characterization is modeled in detail as the data is obtained. We are building novel assay microfluidic systems specifically designed to automate the transport and reaction kinetics data extraction process. We also leverage traditional methods of obtaining these transport data including ultracentrifugation and BIACORE immunoassay.

Recent Accomplishments:

- Two-dimensional on-chip assay combining isolelectric focusing and electrophoresis.
- Developed framework for microsphere bioassays and performed validation experiments.
- Validation of dielectrophoresis and electrothermal models.
- Demonstrated front stacking assay for electrophoresis and validated model.
- Fabrication and test of acrylic microfluidic chips for field-amplified stacking experiments and measurement of kinetic constants using band-crossing (overspeeding) techniques.
- Measured kinetic constants of enzyme/inhibitor system, carbonic anhydrase II and dansylamide, measured using the optical biosensor (BIACORE) and stopped-flow reaction technique.
- Have quantified the reaction kinetics and diffusivity of eight model receptor/ligand systems for validation of novel microfluidic systems and provide typical data to modelers.

Six Month Milestones

- Obtain a series of quantitative ligand/receptor kinetics data using band overspeed technique.
- Quantify performance of two-dimensional assay system.
- Further validate particle physics code including Brownian motion, electrophoresis, and reactions.
- Design, fabricate, and test new integrated fluidic cartridges for concentration assays.
- Conduct PIV experiments for dielectrophoresis particle tracking and slip flow quantification.
- Prototype new microfluidic systems for high-sensitivity and 2D assays.

Team Members and Organizations:

Vinod B. Makhijani and Andrzej J. Przekwas (CFD Research Corporation), Carl Meinhart (University of California at Santa Barbara), David G. Myszka (University of Utah), and Antonio Ricco and Travis Boone (ACLARA BioSciences, Inc.).

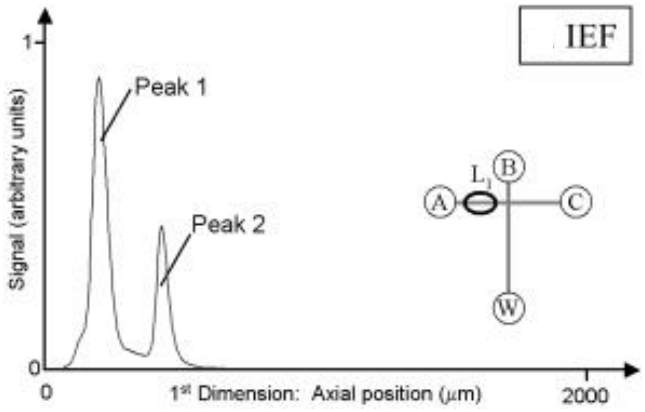

Fig. 1. On-chip IEF experiments: the first dimension of the two-dimensional assay chip. Electropherogram for IEF of a two-component mixture of observed GFP major and minor peaks.

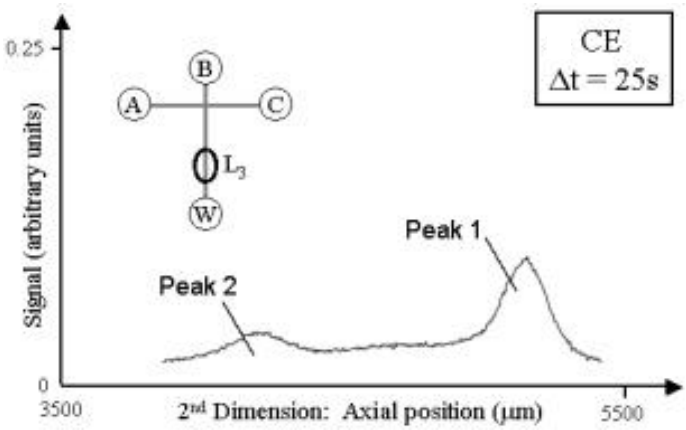

Fig. 2. After the initial IEF separation and concentration, samples are injected into the second dimension to effect electrophoretic separation. Shown is a time-sequence of column-averaged intensity profiles for the second-dimension, capillary zone electrophoresis separation.

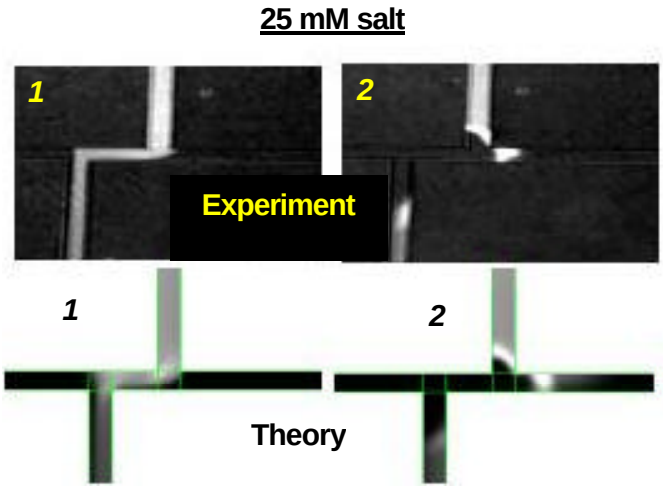

Fig. 3. Comparison of experiment and simulation for back-stacking experiment with and without added salt in the sample.

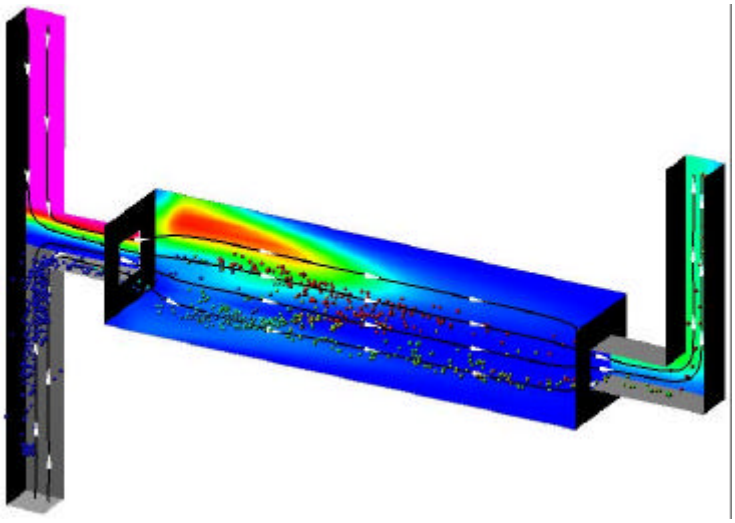

Fig. 4 Microsphere-based assay in a T-Junction biosensor.

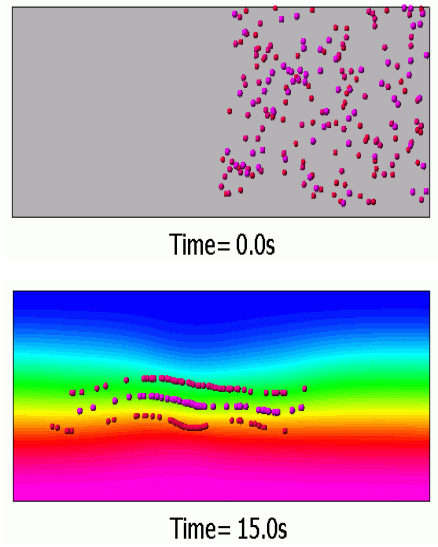

Fig. 5 Separation of Particle Mixture by Traveling Wave DEP in a FFF Device.